CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding at least a functional fragment of a 3-OST protein.

2. An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-OST protein comprising a mature 3-OST-1 protein selected from the group consisting of mature murine 3-OST-1 and mature human 3-OST-1.

3. An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-OST protein comprising a protein selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST.

4. An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a 3-O-sulfotransferase domain of a 3-OST protein selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST.

5. An isolated nucleic acid as in claim 1 wherein said nucleic acid comprises a nucleotide sequence selected from nucleotide sequences within:

(a) SEQ ID NO: 1;

(b) SEQ ID NO: 3;

(c) SEQ ID NO: 5;

(d) SEQ ID NO: 7;

(e) SEQ ID NO: 9;

(f) SEQ ID NO: 11;

(g) a sequence having at least 60% nucleotide sequence identity with at least one of (a)-(f) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and

(h) a sequence differing from a sequence of (a)-(g) only by the substitution of synonymous codons.

6. An isolated nucleic acid as in claim 1 wherein said nucleic acid comprises a nucleotide sequence encoding a polypeptide selected from the group consisting of:

(a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID NO: 2;

(b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4;

(c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6;

(d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8;

(e) residues 66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10;

(f) residues 396-408, 386-4150, or 207-456 of SEQ ID NO: 12;

(g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15;

(h) a sequence having at least 60% amino acid sequence similarity with at least one of (a)-(g) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and

(i) a sequence comprising a chimera of at least two of sequences (a)-(h).

7. An isolated nucleic acid comprising at least 16 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11.

8. A host cell transformed with a nucleic acid of any one of claims 1-7, or a descendant thereof.

9. A host cell as in claim 8 wherein said host cell is selected from the group consisting of bacterial cells, yeast cells, and insect cells.

10. A host cell as in claim 8 wherein said host cell is selected from the group consisting of somatic cells, fetal cells, embryonic stem cells, zygotes, gametes, germ line cells, and transgenic animal cells.

11. A host cell as in claim 8 wherein said cell is a mammalian cell.

12. A host cell as in claim 11 wherein said cell is selected from the group consisting of. COS-7 cells, CHO, murine primary cardiac microvascular endothelial cells (CME), murine mast cell line C57.1, human primary endothelial cells of umbilical vein (HUVEC), F9 embryonal carcinoma cells, rat fat pad endothelial cells (RFPEC), L cells, and cells derived from the transgenic animals of the invention.

13. A substantially pure protein preparation comprising at least a functional fragment of a 3-OST protein.

14. A substantially pure protein preparation as in claim 13 wherein said 3-OST protein is selected from the group consisting of mature murine 3-OST-1 and mature human 3-OST-1.

15. A substantially pure protein as in claim 13 wherein said 3-OST protein is selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST.

16. A substantially pure protein preparation as in claim 13 wherein said functional fragment comprises a 3-O-sulfotransferase domain of a 3-OST protein selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, and ce3-OST.

17. A substantially pure protein preparation as in claim 13 wherein said functional fragment comprises an amino acid sequence selected from amino acid sequences within:

(a) SEQ ID NO: 2;

(b) SEQ ID NO: 4;

(c) SEQ ID NO: 6;

(d) SEQ ID NO: 8;

(e) SEQ ID NO: 10;

(f) SEQ ID NO: 12;

(g) SEQ ID NO: 15;

(h) a sequence having at least 60% amino acid similarity with at least one of (a)-(g) and having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and

(i) a sequence comprising a chimera of at least two of sequences (a)-(h).

18. A substantially pure protein preparation as in claim 13 wherein said functional fragment comprises an amino acid sequence selected from the group consisting of:

(a) residues 21-52, 260-269, 250-276, 53-311, or 21-307 of SEQ ID NO: 2;

(b) residues 21-48, 256-265, 246-272, 49-307, or 21-303 of SEQ ID NO: 4;

(c) residues 42-109, 313-325, 303-332, or 110-367 of SEQ ID NO: 6;

(d) residues 44-147, 351-363, 341-370, or 148-406 of SEQ ID NO: 8;

(e) residues 66-132, 336-348, 326-355, or 133-390 of SEQ ID NO: 10;

(f) residues 396-408, 386-415, or 207-456 of SEQ ID NO: 12;

(g) residues 240-250, 230-257, 23-291 of SEQ ID NO: 15;

(h) a sequence having at least 60% amino acid sequence similarity with at least one of (a)-(g) and encoding a functional fragment having sequence-specific HS binding affinity or 3-O-sulfotransferase activity; and

(i) a sequence comprising a chimera of at least two of sequences (a)-(h).

19. A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides comprising:

contacting said preparation with at least a 3-O-sulfotransferase domain of a 3-OST protein in the presence of a sulfate donor under conditions which permit sulfation of said residues;

wherein, said 3-OST protein is selected from the group consisting of 3-OST-1, 3-OST-2, 3-OST-3A, 3-OST-3B, 3-OST-4, ce3-OST, and conservative substitution variants or chimeras thereof.

20. A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides, wherein said polysaccharides include a polysaccharide sequence of GlcA→GlcNS ±6S comprising:

contacting said preparation with a 3-OST-1 protein in the presence of a sulfate donor under conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S sequence to GlcA→GlcNS 3S ±6S.

wherein the 3-OST-1 protein is selected from the group consisting of murine 3-OST-1, human 3-OST-1, mature murine 3-OST-1, mature human 3-OST-1, a functional fragment of a 3-OST-1 having 3-O-sulfotransferase activity, a conservative substitution variant of 3-OST-1 having 3-O-sulfotransferase activity, and a chimeric 3-OST-1 having 3-O-sulfotransferase activity.

21. A method as in claim 20, wherein said GlcA→GlcNS ±6S polysaccharide sequence comprises a part of a polysaccharide sequence selected from the group consisting of:

(a) GlcA→GlcNS ±6S→IdoA 2S→ GlcNS ±6S;

(b) IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

(c) IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

(d) IdoA→GlcNAc→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

(e) IdoA→GlcNS→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS 6S;

(f) IdoA→GlcNAc 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS;

(g) IdoA→GlcNS 6S→GlcA→GlcNS ±6S→IdoA 2S→ GlcNS;

22. A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides, wherein said polysaccharides include a polysaccharide sequence of GlcA 2S→GlcNS comprising:

contacting said preparation with a 3-OST-2 protein in the presence of a sulfate donor under conditions which permit said 3-OST-2 to convert said GlcA 2S→GlcNS sequence to GlcA 2S→GlcNS 3S.

wherein the 3-OST-2 protein is selected from the group consisting of 3-OST-2, a functional fragment of a 3-OST-2 having 3-O-sulfotransferase activity, a conservative substitution variant of 3-OST-2 having 3-O-sulfotransferase activity, and a chimeric 3-OST-2 having 3-O-sulfotransferase activity.

23. A method as in claim 22, wherein said GlcA 2S→GlcNS polysaccharide sequence comprises a part of a GlcNS→GlcA 2S→GlcNS polysaccharide sequence.

24. A method of 3-O-sulfating saccharide residues within a preparation of glycosaminoglycan or proteoglycan polysaccharides, wherein said polysaccharides include a polysaccharide sequence of IdoA 2S→GlcNS comprising:

contacting said preparation with a 3-OST-3 protein in the presence of a sulfate donor under conditions which permit said 3-OST-3 to convert said IdoA 2S→GlcNS sequence to IdoA 2S→GlcNS 3S.

wherein the 3-OST-3 protein is selected from the group consisting of 3-OST-3A, 3-OST-3B, a functional fragment of a 3-OST-3 having 3-O-sulfotransferase activity, a conservative substitution variant of 3-OST-3 having 3-O-sulfotransferase activity, and a chimeric 3-OST-3 having 3-O-sulfotransferase activity.

25. A method as in claim 24, wherein said IdoA 2S→GlcNS polysaccharide sequence comprises a part of a GlcNS→IdoA 2S→GlcNS polysaccharide sequence.

26. A method for enriching the AT-binding fraction in a preparation of heparan sulfates, wherein said preparation includes a polysaccharide sequence of GlcA→GlcNS ±6S comprising:

contacting said preparation with 3-OST-1 protein in the presence of a sulfate donor under conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S sequence to GlcA→GlcNS 3S ±6S, thereby increasing the fraction of AT-binding heparan sulfates.

27. A method for converting HS^{act} precursor to HS^{act} in a preparation of heparan sulfates, wherein said preparation includes HS^{act} precursor polysaccharides including a sequence of GlcA→GlcNS ±6S comprising:

contacting said preparation with 3-OST-1 protein in the presence of a sulfate donor under conditions which permit said 3-OST-1 to convert said GlcA→GlcNS ±6S sequence to GlcA→GlcNS 3S ±6S, thereby converting HS^{act} precursor to HS^{act}.

28. A method as in any one of claims 19-28 wherein said sulfate donor is PAPS.

29. A non-human animal model, wherein a genome of said animal, or an ancestor thereof, wherein said recombinant construct has introduced a modification into said genome, said modification selected from the group consisting of insertion of a nucleic acid encoding at least a functional fragment of a conspecific wild type 3-OST protein, insertion of a nucleic acid encoding at least a functional fragment of a transpecific allelic variant of the 3-OST sequences, insertion of nucleic acid encoding at least a functional fragment of an allelic variant of 3-OST sequence, inactivation of an endogenous 3-OST gene, and insertion by homologous recombination of a reporter gene coupled to 3-OST transcriptional elements.

30. An animal as in claim 29 wherein said modification is insertion of nucleic acid encoding at least a functional fragment of wild type 3-OST selecting from the sequence consisting of the SPLAG-domain, the cysteine-binding peptide loop, and the ~260 residue ST domain.

31. An animal as in claim 29 wherein said animal is selected from the group consisting of rats, mice, hamsters, guinea pigs, rabbit, dogs, cats, goats, sheep, pigs, and non-human primates.

32. An animal as in claim 29 wherein said animal is an invertebrate.

33. A method of producing antibodies which selectively bind to a 3-OST protein comprising the steps of

administering an immunogenically effective amount of a 3-OST epitope to an animal;

allowing said animal to produce antibodies to said epitope; and

obtaining said antibodies from said animal or from a cell culture derived therefrom.

34. A substantially pure preparation of antibody which selectively binds to an epitope of a 3-OST protein.

35. A substantially pure preparation of an antibody as claimed in 34 wherein said antibody selectively binds to at least a fragment of 3-OST.

36. A cell line producing an antibody of any one of the claims 34.

37. A method for identifying compounds which can modulate the expression of a 3-OST gene comprising steps of

providing a cell expressing a nucleic acid under the control of a 3-OST regulatory sequence;

contacting said cell with at least one candidate compound; and

assaying for a change in the in the expression of said nucleic acid.

38. The method of claim 37, wherein said nucleic acid comprises a marker gene and a 3-OST gene

39. The method of claim 37, wherein said said assaying step comprises detecting a change in 3-OST mRNA level.

40. The method of claim 37, wherein said said assaying step comprises detecting a change in 3-OST protein encoded by said nucleic acid.

41. A method of determining partial sequence information for complex polysaccharides comprising the steps of:

contacting a first sample of polysaccharide with at least one ligand which binds polysaccharides in a sequence specific manner;

contacting the resulting polysaccharide-ligand complex with at least one agent that modifies complex polysaccharides;

contacting a second sample of polysaccharide with the same modifying agent;

comparing said first and second samples for ligand-specific inhibition of modifications caused by said modifying agent.

42. The method of claim 41, wherein said complex polysaccharide is a glycosaminoglycan.

43. The method of claim 41, wherein said ligand is catalytically inactive.

44. The method of claim 41, wherein said ligand is an inactive 3-OST.

45. The method of claim 41, wherein said agent that modifies polysaccharides is selected from the group consisting of epimerases, lyases, sulfotransferases, N-actyltransferases, N-deacetylases, epimerases.

46. The method of claim 45, wherein said modifying agent is a sequence specific degrading agent.

47. The method of claim 45, wherein said modifying agent is a non-sequence specific degrading agent.

48. The method of claims 46, wherein said degrading agent is a lyase.

49. The method of claim 47, wherein said non-sequence specific degrading agent nitrous acid.

50. The method of claim 45, further comprising affinity purifying said modified first and second samples.

51. The method of claim 45, wherein the step of comparing includes a comparison of size profiles.

52. A method of determining partial sequence information for complex polysaccharides comprising the steps of:

contacting a first sample of complex polysaccharides with a 3-OST protein in the presence of a sulfate donor under conditions which permit sulfation by said 3-OST;

contacting said first sample and a second sample with at least one enzyme which cleaves polysaccharides in a sequence-specific manner;

determining the size profiles of the resulting fragments.

53. The method of claim 52, wherein the determining the size profile step further comprises the step of comparing said first sample to a second sample cleaved by the same enzymes.

54. The method of claim 52, wherein said enzymes which degrade polysaccharides in a sequence specific manner are selected from the group consisting of polysaccharide lyases, heparinase I, heparinase II, and heparinase III

55. A method of determining partial sequence information for a sample containing complex polysaccharides comprising the steps of:

contacting said sample of polysaccharide with a 3-OST protein which lacks enzymatic function with a under conditions which permit said 3-OST protein to bind to said polysaccharide in a sequence specific manner;

applying said sample to an affinity column;

applying degrading agents to said column;

analyzing the resulting degradation products.

56. The method of claim 55, further comprising repeating the steps applying degrading agents and analyzing using a series of different sequence specific polysaccharide cleavage enzymes.

57. An isolated nucleic acid comprising a genetic regulatory squences of a 3-OST operably joined to a marker gene.

58. A host cell transfomed with the isolated nucleic acid of claim 57, or a descendent thereof.

59. A method of identifiing compounds capable of modulating the expression of a 3-OST comprising contacting candidate compound seith the transformed host cell of claim 58 and assaying for changes in expression of said marker.

60. A method as in claim 59, wherein said regulatory sequences comprise the 5' untranslated region of SEQ. ID NO: 16.